

V mapping.

July 12, 1948.

(1) W413 x Y64

413 mg. Good yield!

(2) W416 x Y64

on B, + T (C).

OK!

(3) Y87 x W401.

(4) W415 x Y10.

2 taken from B. Pick large colonies to water; small cols. to EMS'. 66 small: 82 large noted. Test T1, T5.

Large: EMS. -R -S +R +S.

Small: 8-: 40+.

-:	T1h	T5	T1
+:			

251-1: many colonies were radially netted, suggesting segregation. On first subseq. streaking, both +, -, and radial streaking were noted. In 1st plate; test + and - both a + and - were T₁ (S).

Restreak from broad streak of 1st plate:
251-2.

July 13, 1948

58-161 37 plates 6 sec. rather smeared but estimate ca. 7000 tested.

Nutrient Agar + 1% lactose + 50 mg/l. T2. Autoclave together

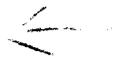
- | | | | |
|----|---|------------------------|--------|
| 1. |  | +++ and slow | W- 426 |
| 2. |  | + and - | 427 |
| 3. |  | + and - | 428 |
| 4. |  | + and - | 429 |
| 5. |  | + and - (fairly slow). | 430 |

July 15, 1948. T2 Glu run.

100 plates x ca. 150 cols./plate = 15000 tested.

4 mutants recovered + tested to be $V_1^{S!}$, Lac- (? for 433)

Take Partially "typed"
section of 24961 from E 1756ae/T1.
and S.O. 254-1.
"partial lapis" in thick section.



(A) Phosphine "GNR" received from Amer. Cyan. Co. Made up to 1mg/ml and filtered through paper. Add to Nutri Bath + autoclave. Add to make conc. indicated in v/ml:

SW7:	10	A7:1 No appreciable growth inhibition noted. Use 100x level for further expts.	Fidelity may be due to eye. Use 10x level.
	20		
	30		
	50		
	80		
	100.		
	0.		

SW10. 10. A10:1

(B) Potassium arsenite, Meeks, made up to 4/100 (as $KAsO_2$)

SW7 1:100 some inhibition B7:1
1:50 appreciable "

SW10. 1:50 " " B10:1

Use 4/10,000 = 1/250 in further expts.
Wash cells for all transfers.

(A) 7:1 is first tube recorded on 253, etc.

P15: Transfer from :2 to :3, loopful transfer.

A10-5.

10 tested 9 carry phage.
1 ? Repeat test.

A7-5.

16 cultures tested on 5436. & SW-10
all still carry phage.
> 2 are not phage.

July 16, 1948.

Grow W-252 and W-327 in Lysa broth overnight.
(Test first on Lac + Mal EMB, T2).

	EMB/Lac	EMB/Lac	EMB/Mal	T2 Lac	T2 Mal	
252	-	++ (1-noted)	-	+++	+++	* all white!
327	-	-	±	±	+++	

purify + restreak. ~~Irradiate 10 plates each of T2 Lac + T2 Mal with 252 + 327 respectively.~~

Irradiate suspension of 252 Lac + on EMB + T2, pure plates each.
Controls: EMB: all ~~++~~ +++.
T2 " "

- EMB:
1. Small - ? large + small S.O. on EMB. all+
 2.  + and - W436
- T2
3.  + and slow
 4.  slow +
 5.  all - W437
 6.  + and slow
 7. - colony noted on original streaking of W-252. = W431

19. Saturated gives colonies with a
strong -) reaction on T2. Purify and
keep as W-462.

July 19, 1948.

Quadrant W252, purified, br. sec. on a) ~~EMBLac~~ 45 plates

b) T2 Lac 45 plates.
ca 200 pu = 9000.

D.G. fecit

W327 " 6 secos.

on a) EMB Mal } 45 plates.
b) F2 Mal } 200 pu. = 18,000.

W252). b). S.O. from T2 to EMB Lac.

- | | | |
|-------------------|-------------------|-------------------|
| 1. slow | 13. + and - 448. | 31. + + - 458 |
| 2. slow | 14. all - 449 | 32. + + - 459 |
| 3. slow | 15. all + | 33. mostly - 460 |
| 4. slow | 16. all - 450. | 34. mostly - 461 |
| 5. + and - W-438 | 17. + and slow + | |
| 6. slow. | 18. + and - 451 | |

1 plate {

W327). b).

- | | |
|------------------------|---------------------------|
| 1. - or slow. W439. | 19. all + S.O. on T2. |
| 2. + and slow | 20. + and - 452. |
| 3. + and - or s. W440 | 21. slow + small |
| 4. mostly - . W441 | 22. " " |
| 5. all + | 23. mostly - ; some + |
| 6. + and slow | 24. slow + small |
| 7. + and slow 442 | 25. (temperature?) all + |
| 8. all +. | 26. - (slow ±?) 453. |
| 9. +, -, and slow | 27. all - 454 |
| 10. + and slow | 28. - or s. + 455 |
| 11. + and - 446 | 29. + and - 456. |
| 12. + and slow 447 | 30. + and - 457 |

EMB.

All cultures tested: see top - 1/5

July 16, 1948.

Prepare N.A. plates \pm 2% sucrose + 50r/ml T2 + varying
Tergitol 7 (~~in ml~~) in ml/50 of .1% solution:
N = - sucrose S = + sucrose.

P18: Tergitol	N	S.
.2	Mod growth $\frac{1}{2}$ plate	heavy growth + conidiation
.5	"	"
.7	no growth	* slim. growth + conidiation
1.0	1cm. thin growth	Moderate growth to edge of plate
1.4.	< "	No growth

No plates showed colored mycelia.

Next day: growth similar + advanced

No color.

July 20 ff.

SW7/6 purified from 254 residues following individual colonies.
High mutation rate from R \rightarrow S apparent.

July 19. S.O. SW7/6. Test 20 colonies on Sp 6.

19 R
1 S.

1 R inoculum for cross

July 22, 1948. SW7/6 X SW10

Gen T(10):

SW7

SW10 = Tr - Ar + Sp6^S $\xrightarrow{R.M.}$ Tr + Ar - Sp6

SW7/6. IL - Ar + Sp6^{R \rightarrow S}

also S.O. parental suspensions
as USA to check stability.

July 25, 1948.

SW7. No cols 1/2 pl.

SW7/6 " 1/2 pl

SW10 2 cols 1/2 pl. \rightarrow

10 X 7/6 9 cols 1/2-3 pl. Test \rightarrow 9 cultures.

#5 Ar + Sp6^R

#1-4, 6-9. Ar - Sp6^R.

Repeat phage tests on T(10) \bar{c}

S71 control. Checks on fermentation
of Mal, Lac + Gal.

All sensitive!

Contn. 251.

Test five "±" colonies from 251a for mutation

±	1	0	BM	TLB, BM TLB,
	2	+++	+++	+++
	3	"	"	"
	4	"	"	"
	5	++	"	+++

Lact	1.	-	+++	-	+++	BM!
	2.	-	"	-	"	BM.

vac (251-6) → MTLB² W472
 → do start for subculture.

vac	1.	-	-	-	+++	TLB, BM?
	2.	-	-	+++	+++	TLB.

TS S!

When first tested, with right
 missense, was T-LB. Recheck for
 a better requirement.

"±" colonies seem to be prototrophic, and are splitting off numerous
 recombinant types. Strike out tubes of ± / BM TLB,
 and test colonies for all nutritional and phage characters available.

P24. (1)-(2) streaked out from BM TLB, is vac E415. Test mutation
 of single + and a single - from each:

	0	BM	TLB ₁	Com.	TS
2. 1-			+++	+++	R
2. 2-			+++	+++	R
2. 3.		+++		+++	S
2. 4.	-	+++	-	+++	S
2. 5.			+++	+++	R
3. 1+		+++			S
3. 2+		+++			S
3. 3+		+++			S
3. 4+	+	+++	+	+++	SR
3. 5+		+++		+++	S

-S
+R.

is vac -
 Note! of vac -, a
 recombinant.
 ↓ W-4/66

July 23, 1948.

- (A) 847 / Galactose EMB. 6 sec. Hanovia lamp.
 31 x 300+ readable plates (many others smeared). ca 10,000.
 11 possible tested. 260-A: 111. 1 Gal - found SW-13.
 Check 0 Sp-6.

- (B) 161 / Glucose T2, EMB. 45 }
 45 } x ca. 300 each.
 many smeared.
 T2. 3 tested. 1 + and -
 260-1. Recheck and test on Lac, T1.
 Lac - T1^S W-467

July 23, 1948.

S.O. from 251a1 to EMS. Predominantly lac + prototrophs (1:100 or -). Pile 28 of these and streak out on lac EMS, P.V. Same suspensions!

Designate mosaic + as M.
Write types in relative order of frequency.
() v. varying.

P25.

1. M - +

2. M - +

3. M + (-)

4. M (-) (+)

5. M

6. M -

7. M (-) (+)

9. M -

10. M + -

11. M - (+)

12. M - (+)

13. M + (-)

14. M (-) (+)

15. M (-) (+)

16. M.

17. M (+)

18. M (-)

19. M

20. M (-) (+)

21. M - (+)

22. M + (-)

23. M - +

24. M -

25. M - (+)

26. M - +

27. M - +

28. All -.

Streak out on ~~lac~~ EMS.

a) M colonies

b) equally dense mixtures of - and +

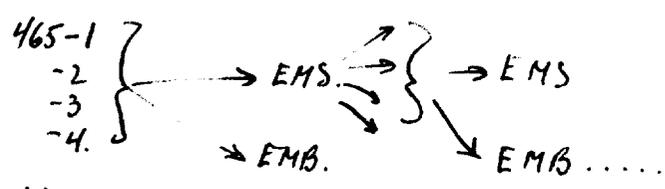
Streak out on EMS: M colonies.

Test for sensitivity.

Suspensions 1-9 were tested with T1 and T5 for sensitivity to T1 + T5 on T(0). Each culture was sensitive to both phages. From this T(0) plate, inoculate T(0) slants as W465: 1-9. (H for heterokaryon).

July 25, 1948.

PLAN: streakout in series



to indicate

whether 465 can be "purified".

P25. Streakout -1, -2, -3, -4. (from T(0) phage test plate: see 261.)

- A. EMS' Numerous colonies, all +. on all 4
- EMB. +, -, and M colonies predominating.

A27. S.O. 4 Colonies from A1.

EMS 4 cols from B1. →

- B. EMS. 4 +, - and M predominant.

C. EMS. All four are +

P28 EMB. + and -; too thin to determine whether they are mosaic.

Take 1 col. each from C for D ↓.

P30. D: EMB. P31. Most colonies still mosaic.

- EMS. (A1) ① 1 + colony with - sector. Others (-)
- ② all +.
- ③ all + → E(1-4).
- ④ 1% -; others +.

P31. + colony to T(0) liquid.
 grow overnight: streakout
 on EMBs 262 - D11
 ca 60% variegated. Numerous
 + colonies.

P1. EMS. 1,3,4 all+ 2 1:100, -:+

EMB. All predominantly variegated. Select four colonies from "4" for

F ↓

Aug. 12, 1948.

J. EMS: 1-4 All +EMB: $\left. \begin{array}{l} 1 \\ 2 \\ 3 \\ 4 \end{array} \right\}$ mostly Var.EMB + Na. nucleate $\left. \begin{array}{l} 1/2\% \\ 10\% \end{array} \right\}$ variegation, uncreasable due to modificationEMS. All+. ^{EMB.} All Var.K. ~~EMB~~P14. EMS 1-3. All + 4. 1-. 2 cols suspended for M ↓

L. EMB All V.

P16. (M). EMS 1, 2 All + EMB Varieg. Store in ref.

P23+ (N). do. Store in ref. P28 +.

9/10 ca. 0. do. from EMB plate to EMS for P, 9/20/48.

Verify colonies on EMS + transfer to T(0) agar as W465
282 P

August 3+, 1948.

F. EMS. All 4: all+. 4 cols. from ①
EMB. all predominantly variegated.

G. EMS A7. All+.
EMB. 1. Predom. Var.
2. " " "
3. Partially Var. Many full+ or sl. varieg. colonies.
4. Predomin. Variegated.

Select 4 colonies from EMS-1 as H: 1-4.

" " " EMS-3 as H: 5-8

A8: 1-8 tested as T1, T5. mEMS; EMS. All 8 were +S on ~~T1, T5~~ EMS.

H. An EMS, all showed ± resistance in *U. vis* assays, T1 + T5 illustrating the segregants.

EMB: 1-8 all prominently variegated.

EMS (A9) 1: appreciable -
2-8 All+.

For I choose 2 cols. (1-2) from 3 and 2 cols from 5. (3-4)

A9. EMS
EMB.

I. EMB.
1 Var.
2 Var.
3 Var. } colonies tend to look uniformly dark when crowded.
4 Var.

EMS. All, all+. 2 from 4 from ③ → J P10.

P10.

J

July 26, 1948.

Sec:	261-	Lac.	0	BM	TLB,	BMTLB, Lac	TI	T5	Recheck wt. reading later idy.
1	7	-			+++	+++	-	R	R
2	7	+		+++		+++			
3	8	-	-	-	-	+++	-	R	R MTLB, ✓
4	9	-			+++	+++			
5	10	-	++	++	+++	+++	-	R	R
6	10	+				+++		-	
7	23	-	-	-	-	++++	-	R	R MTL ✓
8	24	-			+++	+++			
9	26	-	+	+++	+++	+++	-	P ^{R?}	R ^{S?} mixed?
10	26	+		+++		++++	+	P	S. parental. MTL

259-6.

12
13
14

Test for phage and streak on lac E M₁₃ from BMTLB, tube.
Repeat mutation of 3 + 7 directly.

263: Test - segregants.

R: HTL - 15 TL - ~~10~~¹⁴ Pectroph. - 1
 T - 2 M-1 ML 2 MT 1.

S. M 6.
 O 2.

+

R. TLB, (M?) 1.

S. M 8.

M is definitely not segregating properly, being in marked excess both in lac⁻ and lac⁺ categories. Is it sorting properly? However, this may not be a random sample. B₁ + B₂ certainly are not.

Save as

		(H5)	
W-472.	M-T-L-	lac-R.	= 259-6.
473	M-	lac-R	
474	M-L-	lac-R	
475	M-T-	lac-R	
476	T-	lac-R.	
477	T-L-B ₁ -	lac-R.	} (for further crosses).
478	M-	lac ⁺ S	

Retest single colon

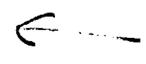
		-T	-B	-M	-L	+	
		BMTL-B,	BMLB,	MILTB,	BTLB,	BMTB,	BMTLB,
U463	3	-	-	+++	-	-	+++ MTLB,
W467	7	±	-	+++	-	-	+++ MTL(B,?)

	0	BM	TLB,	BMTLB,
{ 5a	-	-	-	+++
{ 5b	-	-	-	+++
9a	-	++	-	+++
9b	-	++	-	+++
10a	-	+++	-	+++
10b.	-	+++	-	+++

sublac -
~~parental~~. Check
 phage. ~~Understandably~~

parental in all respects.
 i.e., BMlac + V₅^S · V_{1c}^R

Pick 45 prototrophs at random from EMS.
and test for phage sensitivity to T5.



lac- (4 colonies) 4 S 0 R.

lac+ (41 ") 37 S. 4 (?) R.

Recheck + of. 4 S's. all were S.

all + prototrophs → primarily M colonies, with poorly demarcated sectors. (Also occasional + and -

(The plating of 261-1 [→] has given the most sharply sectorial colonies noted so far).

Search for symcayon:

204.

w-1 x Y40.

July 27, 1948.

Cross heavy suspensions of w-1 and Y40 on EMA(0) Malt.

Pu plate,	-	+
P28:	26	2
	17	2
	13	5
	15	0
	16	1
	8	0
	8	0
	11	1
	18	1 + 1?
	15	1
	17	2
	5	1 SEC.
	14	1
	11	0
	22	3
	22	6
		21

Pick all +'s and a) streak out on LacEMB b) test with T1 on EMS.
 4 +R 6-S 6-R. No +S (possible heterozygote).

A29. New crop of Malt+ colonies (some rather hazy). Pick + test on lac, T1.

14 tested with lac, T1.

5-S 7-R 1 +[?]S Streak out on Lac S +
 Lac EMB.
 269-1. pure lact.

July 26, 1948.

Grow 261-1 in T(0) 24h. Distribute and plate carefully
on EMSB, EMS!

	Total.			
1. EMSB.	14.	3-	2+	9M.
	12	1-	3+	8M
	13	4-	1+	8M
	10	1-	2+	7M
	12.	3-	1+	7M.
	<hr/>			
	61	12-	9+	39 M.

2. EMSB.	21.	4	2
	28	4	2
	17	1	1
	21	4	3+
	27	3	4
	<hr/>		

+1 colony
large.

		16.	12
3.	32	1	4
	33	1	1
	37	4	4.
	35	2	2
	45	6	7

	Total.	#	-	#+	M.
4:	19.		3	1	
smared.	31		0	2	
	25		2	0	
	37		9	0	
	22		2.	3	

Collect +, -, and clearly sectored colonies from these plates.

O = +
S.

O = ~~B~~ - Test on EMB Lac / TS.

Sectored colonies were chosen for complete analysis if they appeared to have segregated early in colony formation.

Pick 4 colonies (A-D) from each set of plates (1-4) + 9.0 on Lac EMB

EMS:	+	-	Total	Mean + prototrophs
1.	7 12 14	0 0 0	7 12 14.	11
2.	15 20 15	0 0 1	15 20 16	17
3.	35 19 34	0 0 2?	35 19 36.	27
4.	23 22 42	0 0 1	23 22 43.	27.

Pida - colonies more or less randomly from 265 plates + test \bar{c}
 TS. Parental Comb. = lac- TS^R; Lac+ TS^S. (letter diff. by M)

lac+ : ~~9R:1S.~~
 9S:1R

lac-	R	S	
	9	1	
	16	4	
	15	4	
	<hr/>		
	40	9	749.
	9	1	
	<hr/>		
	49	9	58.

Ca 20% of the lac-
 signants are non-parental.
 Ca 10% of the lac+ sign. are
 non parental.

July 29, 1978.

- 1A: 1-9 Lac- 10 Lac+
- 1B: 11-7 Lac- 8-10 Lac+
- 1C: 21-25 Lac- 26-30 +
- 1D: 31-35 - 36-40 +
- 2A: 41- -50
- 2B: 51- -60
- 2C: 61- -70
- 2D: 71- -80

B- and B₁- have been scoring v. poorly indeed + should be omitted from consideration

parents were M-Lac+ V₅^S
T-L-Lac- V₅^R.

Test sensitivity to TS:

	1A	1B	1C	1D	2A	2B	2C	2D
	0	10	20	30	40	50	60	70
1	R	R	R	S	R	R	S	Lac- R +++
2	R	R	R	S	R	R	S	R
3	R	R	R	S	R	R	S	R
4	R	R	R	S	R	R	S	R
5	R	R	S	S	R	R	S	(S)
6	R	R	S	S	S	S	S	R
7	R	- R	S	S	S	S	R	S
8	R	+ S	S	S	S	S	R	R
9	- R	(R)	S	S	S	S	RS	R
10	+ S	(R)	S	S	S	S	S	(Lac+ R. Th.)

Nutrition: 1 (MTLB₁) (M) TL (M) MTL TL(B.) L (+++)

10. M +++ M(T?) M TL T/L TL TL

10 of 11 subcultures completely ~~empty~~, 21, 51, 10, 50, 60 = 5 were parental. i.e., had no cross with M and TL.

W-471.

July 30, 1948.

Retest cultures 71-80 nutritionally and for lac; phage, from phage test plates. Preserve 2D mixture on slant as 265-2D.

Repeat phage.	Lac	TS	Nutr.	Graph ⁺	Lac	TS	Nutr.
71. -R	-	R	MTL	✓	+++ G	61. -S	M
72. -R	-	R	TL	✓	M	-S	M MTL
73. -R	-	R	MTL	✓	+++	-S	+++ MTL } do do
74. -R	-	R	TL (15.)	✓	M	-S	L ✓
75. -S	-	S	M	✓	TL	-S	L ✓
76. +S; -R	+	R	+++	✓	+ S	+ S	M ✓
77. +S	+	S	M MTL	✓	+S; -R	+ R	+++ ✓
78. +S; -R	+	R	+++	✓	+S; -R	+ R	+++ MTL } heterozygote
79. +S; -R	+	R	+++	✓	+S; -R	+RS	+++ MTL
80. +S; -R.	+	R.	+++	✓	+S. ✓	+S.	M MTL

Phage tests n.g. Repeat!!
Do. 61-70. Repeat phage tests.

Many of the Lac+ recombinants are apparently still heterozygous in these platings, especially if prototrophic. Perhaps they have a lower segregation frequency. Struck out #78 and #88 on EMSlac
See 271

These colonies obviously have more than 4 kinds of recombinants

July 28, 1948.

Grow SW10 (Tr-Ar-) and SW13 (IL-Gal-) in ~~YB~~ YB overnight,
wash + plate conc. suspensions on T(0) plates.

Pr8. 10: (3 plates). No cols.

13: 3 plates No cols.

X: 7 plates. Syntrophic background + a scattering of tiny
colonies. Pick same + streaks out on T(0).

1.

2.
3. 3 tested on gal; arab.

No exchanges.

1: Gal - Ar +

7: Gal + Ar -

A29. Pick 9 further cols + test:

9 tests: all Gal + Ar -

Summary: 16 Gal + Ar -
1 Gal - Ar +

From exp. 265, pick variegated colonies, streakout & recover 1+ and 1- from each variegated. Align as far as possible (some plates had no well correlated +s so that the -s are unpaired). a - b = +.

	Lac	T5	Nutr. (Lig.)	Agar.		1-20 are from earlier streakings.
1 a	-	R	M+++	B,+		
b	+	S	M	B,+		
2 a	-	R	MTL ...	B,-	M+ } 5	
b	+	S	M	B,+	M- } 14.	
3 a	-	R	M-	B,-		
b	+	S	M	B,+		
4 a	-	S	L TL ident	B,-	R+ } 7	
b	+	S	M	B,+	S- } 11	
5 a	-	R	u.g. M	B,+		
b	+	S	M	B,+	T+ } 14	
6 a	n.g. +		M(L)	B,+		
b	n.g. +		M	B,+	T- } 5	
7 a	-	R	M ...	B,-		
b	+	S	M ...	B,+	L+ } 13.	
8 a	-	S	TL ident	B,-		
b	+	S	M	B,+	L- } 6	
9 a	-	R	TL	B,+		
b	+	S	M ...	B,+		
10 a	-	R	TL	B,-		
b	+	S	M ...	B,+		

In this series, liquid nutritional tests covered only MTL due to the failure of B + B, to score & present washing facilities.

Every + in this series is M- Lac+ V₅^S

The "-"s are: -S:2 -R: , with a variety of nutr. requirements.

Preserve (2a).

	A.		B.	
	lac	TS	TS	ML
21.	+	S	±	R.
2	-	R	space.	+
3	-	R	+	S
4	-	R	+	S
5	-	R	+	S
6	-	R	+	S
7	-	R	+	S
8	-	R	+	S
9	-	R	+	S
30	-	R.	+	S

	A		B	
31.	-	R	+	S
2	-	R	+	S
3	-	R	+	S
4	-	R	+	S
5	-	R	+	S
6	-	S	+	S
7	-	S	+	S
8	-	S	+	S
9	-	R	+	S
40	-	R.	+	S

	A		B.	
41.	-	R	+	R
2				
3				
4				
5				
6				
7				
8				
9				
50.				

51.	-R	+S
52.	-R	+S
53.	-R	+S
54.	-R	+S
55.	-R	+S
56.	-R	+S
57.	-R	+S
58.	-R	+S
59.	-R	+S
60.	-R	+S.

61.	-R	+S
2	-R	+S
3	-R	+S
4	-R	+S
5	-R	+S
6	-R	+S
7	-R	+S
8	-R	+S
9	-R	+S
70	-R	+S.

phage? ↑

11	-R	+S
2	-R	+S
B	-R	+S
4	-R	+S
5	-R	+S
6	-R	+S
7	-R	+S
8	-R	+S
9	-R	+S
20	M -S	+S

Of ^{100.} ~~80~~ acceptable tests, 5 recombinations between lac and ~~ts~~ H_s.

	A	B		A	B		A	B
71	-R	+S	81	-R	+S	91	R	+S
72	-R		2	"	"	2	R	+S
73	-R		3	"	"	3	R	+S
74	-R		4	"	"	4	R	+S
(75)	-S	M	5	"	"	5	R	+S
76	-R	?	6	"	"	6	S	+S
77	-R		7	"	"	7	S	+S
78	-R		8	"	"	8		+S
79	-R		9	"	"	9		
(80)	-S	M ↓	10	"	"	10		

101	-R	+S	111	-R	+S	121	R	+S
2	-R	+S	2	-R	+S	2	R	+S
3	R	+S	3	-S	+S	3	R	+S
(4)	-S	+S	4	-S	+S	4	R	+S
5	-R	+S	5	-S	+S	5	-R	+S
(6)	-S	+S	6	-R	+S	6	-R	+S
7	-R	+S	7	-R	+S	7	-R	+S
8	-R	+S	8	-R	+S	8	-R	+S
9	-R	+S	9	-R	+S	9	R	+S
110	-R	+R	130	-R	+S	130	-R	+S

130		
131	-R	+S
2	-S	+S
3	R	+S
4	-R	+S
5	-S	+S
6	-R	+S
7	-R	+S
8	-R	+S
9	-R	+S
140	-R	+S

Total: among ca 155 } lac - ¹⁴ ~~14~~ recombinants. (-S)
 135 } lac + 2 recombinants (+R)

Many of the - cultures of the preceding series are somewhat densely papillate, suggesting they may be *mysine*. Re-purify the following as Lac-⁺ recombinants.

4a, 8a, 21a, 36, 37, 38, ~~39~~³⁰, 75, 80, 96, 97, 104, 106, 110, 113,
132, 135 (a).

68, 110, (b).

Nutritional Tests.

On liquid:

W447	TLB.
W448	M.
W-1/1	TLB.
W21.	TM! ?

	Lac	T5	Nutr. (liquid).	✓
132a	-	S	M	
113a	-	S	M	
37a	-	S	M	
38	-	S	M	
20	-	S	M	
106	-	S	M	M
133	-	S	M	M
96	-	S	M	M
80	-	S	M	M
75	-	S	M	M
W-478	+	S	M (w-1)	M-
110B	+	R	TLM	TLM (b, b, ?)
63b	+	S	M	M-
36a	-	S	M	M-
21	-	R	M	M-L-
8	-	S	M	M-
4	-	S	M	M-
110	-	R	TLM	T-L-
104	-	S	M	M-
97	-	S	TLM.	M-
W-21.			M-	

A.

B.

See 274.

July August 1, 1948.

Cross, heavily, W477 x 478 on EMS lac agar (- thiamin) for lact + combinations.

A4: Occasional + colonies; no - noted at this time ca 2-3/plates.

29 + tested all TS^S on EMS. However, all but "8" are apparently pure + when streaked out on EMB. 267-8 shows marked variegation S.O. on EMB, EMS + transfer to T(0) as W-~~472~~ 479

A) Single colonies from 1-29 were picked and streaked for test on TS on EMB + EMS. These plates were inadvertently refrigerated until P7 when they were incubated.

B) Streaks from A4 TS-test plate were picked for ~~re-~~ retesting on TS, EMB + EMS.

A: EMB: +S. No - residue suggesting segregation.

B. ditto. All seem to be stable +S. This is incredible in terms of linkage hypothesis. Save 1-5 as 267:1-5 for further study later.

August 2-3, 1948.

	W-470		W-108		58-161	
Gluc	++	A+G	-	A+C	+++	A+G
Lac	-	-	-	-	"	A+G
Mal	-	-	-	-	"	A+G
Tre	-	-	-	-	++	A+G
Sac	⊕	-	+	A+G	+++	A+G
Gna	+	A+G	+	H+G	"	H+G
Arab	+	A+G	+	A+G	"	A+G
Xyl.	+	A+G	+	A+G	"	A+G
Fru	+	A+G	-	A+G	"	A+G
Heum.	+	A+G	-	A+G	"	A+G
Rham		A+G		A		A+G

Tests 16h. fermentation tubes.

W-470 " W-108 "

August 3, 1948.

- P2. 1 colony from 262E (synth) inoculated in T(0). Stk's overnight.
- 10 A3. Transfer .5 and 1.0 ml to 10ml fresh T(0) and shake.
- 9 picked by Di. The Cory to a tryptone broth; None grew. Expt N.G.

August 3, 1948.

Use same inoculum as in 269. (Washed)

broc. .5 ml into each of following: (additions / 10 ml + conc) *Ulothrix*
 Turbidity 8P4: etc. TLB, BM.

1. Basal (see infra) - phosphate		18	22
2. " + .05 ml "		29	42
3. " 0.1 " "		35	45+
4. " .5 " "		48	75
5. " 1.0 " "		43	96
6. " + .5 ml P. + 5% Na nucleate	3 (deposit on bottom)	9	several
7. " " 2%	11 (extended.)	15	
8. " " 1%	21	57	
9. " " .5%	27	63	
10. T(10)	60	87 (colored).	

11. Lemnassay broth.

12.

H₂O 4. 2-
 broc. 14 14
 Standard A. = 100.

Basal = 1 l.

de Columbia p. 109 ff.

Na acetate
 KNO₃ 1
 NaHCO₃ .5
 Na citrate .2
 Am. sulf. 2
 Mg SO₄ .1
 CaCl₂ 4.
 Glucose 5

phosphate solution class:

30g K₂HPO₄ / l. = 10mg P/cc.
 10g KH₂PO₄ / l.

Analyse out cultures from: (1), (3), (5), and (9, 10, 11).

v	1	3	5	9	10	"
+	26	21	5	1	6	"
+	4	6	11	4	4	could not be used!
+	5	4	15	mostly - or +		

Aug. 1-3, 1948.

Ref. 265c.

265-68 and 265-78 are derived from single, apparently pure, + colonies which behaved a) phototrophically and b) on lac T5 broke up into +S and -R. Streaked out on A) lac EMB and B) lac EMS.

A). Pick single + colonies and test on T5 on EMB and EMS.

EMB: 10 cols. - 68 all + R. Retest!
 - 78 "

EMS: none grew.

B) Scattering of + phototrophs is rare -. Pick +'s and a) streak out on EMB b) test on EMS-T5 c) on EMB T5.

D + c: b. all were + S. c) all reacted + R.

a) AY: seem to be segregating typically i.e. +, - and Nancy; predominant

Production of heterozygotes.

Aug. 6, 1948.

① 477 x 478 - lac EMS.

② 477 x W-21

③ 478 x W-1/1 (mMal EMS)

3M + 4M n.g. background too heavy

④ W21 x W-1/1. (mMal EMS)

P.S. ① 9 plates. ca 8+ : 4-.

Pick + cols. + test for T5 resistance on EMS lac'. Also, S.O. on EMB. ~~→~~

②. 9 plates lac EMS. ca 7- No +! Pick one possible slow + on lac + Mal EMB → is (-) on lac S, and show a few + on lac EMS. No Maltoz.

③. 8 lac S plates.

+	-	+	-
9	10	10	5
3	4	22	15
3	10	8	11
4	5	6	5
		4	2
<hr/>		<hr/>	
19.	29	50	38
		788.	

Test on lac S for T5 and S.O. on Mal EMB!

①. 2 n.g. 1, 3-7 tested: all lac+, 1/5^S on lac EMS!

② None of these show signs of reversion when streaked out on EMB lac!
 (A9) → 5 additional + and -.

③. 79 tested: 17 is -S; All +s are T5^S! Streak out on Mal EMB: #1 is Mal+! others are Mal-. Streak out #1 ^{on lac} and #4, + #7 as possibly lac± from appearance of phage plate.

1. 2 n.g. 1, 3-7, all +S #4 is unmutated, lac- and some marginal of colonies. 152.

P.O. #7 is distinctly variegated. S.O. on Mal. + lac EMB.

Aug. 11, 1948.

See 272 last p.

W482 (on colonies on Mal EMB: all -
W483)

On Lac EMB: Most colonies were + or -, occ. Var.

- 482: 1
- 2
- 3
- 4.

483 - showed more frequent variants.

Talae lac + prototrophs from 8/9 plate on Lac S 273-3-4
and 273-3-1.

- 482: {
- 1. +, - and V
 - 2. Mostly V.
 - 3. + - and V.
 - 4. Mostly V.
- E4B {

Picky to T(0) as W482.
from EMS.

- 483. 1. +, - and V
- 2. Mostly V. → W483.
- 3. Mostly V
- 4. (EMB) - .

(3). 51 additional Lac+ tested on MalEMB - TS.

A10.

8 were appreciably Mal+. All apparently TS^R, streak there out as 272a 1-8. Parents were checked:

w21	Mal -	V ^S	& QK.
w477	Mal+	VR	
w478	Mal+	V ^S	
w480	Mal -	VR	

40 Lac- tested: 3 possible Mal+ noted. 2^S: 1^R.
S.O. as 272a 9-11.

- 9. Pure Mal+
 - 10. Mal- and +; nonvariegated cols.
 - 11. Pure Mal+.
- } on Mal EMB.

On Lac EMB.

- 1. Occ. Var. colonies. streak to MalEMB, LacEMB + see EMS as w484.
- 2. + and -
- 3. Pure +
- 4. + and -
- 5. + and -
- 6. + and -
- 7. Pure +
- 8. - and Var. As (w485).

484 - Pure Mal+ . Lac + and - . LacS not yet ready.
and Var.

485 - Pure Mal+ Lac +, - and Var. " "

486 - Mal+ or ± Var, + and Lac - " "

Aug. 13-14.

Isolate + checks W482- W486.

482. 1. Mostly V. 2. + and v. 3. v. 4. V, +.

483. 1. largely V
+ 2. V, +.

3+4 } all +!

484. 1. V. 2. v. 3. v. 4. v.

485. 1. v. 2. v, +. (3 v.) 4. v.

486. (1 v. 2. v, r, - 3. v, v.

272-1 colonies. 5+ 5-(6-10)

- ↳ 1. Mostly -, some + No V.
- 2. " " "
- 3. All +
- 4. All +.
- 5. +, - and Var. Pide as w486 to LacS, LacB, MalB.
- 6. Mostly -, some + No V.
- 7. "
- 8. "
- 9. "
- 10. "

Phage tests on T5 LacS

	var	T5
1	-	R
2	-	S
3	+	S
4	+	S
5	+	S
6	-	R
7	-	R
8	-	R
9	-	R
10	-	R.

no residual film, characteristic of V_{1C}^R

Chemical control of segregation.

August 7, 1948.

Basal medium of 270. \bar{c} 1.5% agar.

Adjust upwards to 7.3 before adding buffer.

1. + M/500 phosphate, pH 7.0. T(0).

2. + " " + BMTLP₁

3. + M/50 " " T(0).

4. " " " "

5. " " " + 1/2% Na nucleate.

P7. Strake out a colony from 262-51 as source of heterozygotes. Also, suspensions of W-477 + W-478.

51 grew rather well on all media. 477 + 478 did not grow.

on 1 or 3. W478 did very well on the other media, and 477 moderately

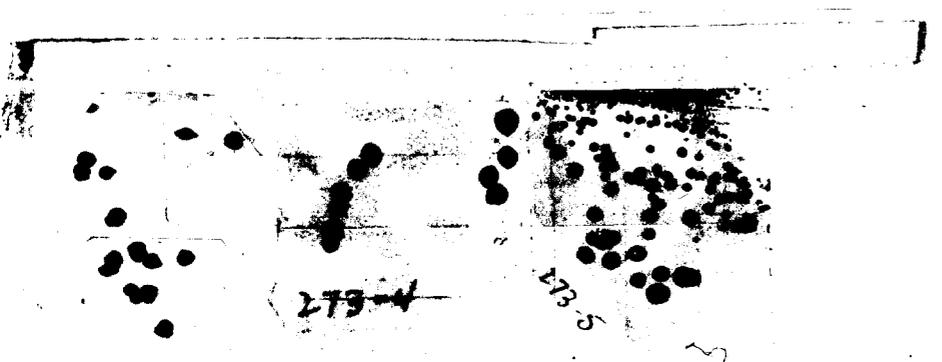
well! Pick 10 colonies each from 3, 4, & 5 + S.O. on Lac EMB.

A10. (1). 1 v. 2 v. 3 v. 4 v. 5 v. 6 v. 7 v. 8 v. 9 v. 10 v. Predominantly variegated.

(2). 1-3. ^{??} V. purum. 4 T, V. 5. v. 6. v. 7. v., 8. v. 9-10 unresolvable.

(4). 1-4 largely + and -, occasionally variegated. 5-8 same. 7-10 same.

(5).



273-1

$PO_4 = M/500$

$PO_4 = M/50$

$PO_4 = M/50$
Na nucleate .5%

August 8, 1948.

S.O. to reify:

~~10~~ (repeat!)

121-130.

	lac	T5		lac	T5	
	A.			B.		
121	-	R	TLB ₁ ✓	+	S	M
2	-	R	M	"	"	M
3	-	R	M	"	"	M
4	-	R	TLB ₁ ✓	"	"	M
5	-	R	M	"	"	M
6	-	S	ML	"	"	M
7	-	R	MLB ₁ ✓	"	"	M
8	-	S	TLB ₁ ✓	"	"	M
9	-	S	TLB ₁ ✓	"	"	M
130	-	R	ML	"	"	M

6, 8, and 9

These were streaked out as lac and individual colonies tested.
 10 colo. each, all were lac- V₅^R! Gf. growth is + tubes!

8/11-12²¹¹

Lac + cont.	-B 12-97	-L	-M	-B ₁	-T	+	All Lac + V ₅	Nutr.	Nutr. var-par.
(75) 8a	+	+	-	+	±	+	S	M	M-✓
25a	+	+	-	+	+	+	R	M	M-✓
37a	+	+	-	+	+	+	S	M	M-✓
38a	+	+	-	+	- +	+	S	TM	M-✓
96a	+	+	-	+	- +	+		TM	M✓
97a	- +	- +	- -	- +	- -	- +		ΔC -	M-✓ TM-
20a	+	+	-	+	- +	+	S	TM	M-✓
104a	- -	- +	- -	- -	- -	- +		TMB.	M-
113a	-	-	-	-	-	-		mag	M-✓

8, 4, 20, 21, 37, 80 r V₅-S

75 V₅-R Reel cube: S.

104 is of special interest.

Aug. 9.

(A) Pick vac + papillae from 266d test plates and 50. ml lac EMBS.
2/struck.

(B) Plate 132a, 113a, + 37a suspensions from BMTLB tubes
in T5 and T6. to pick up resistant.

		Isolated + T5	Nutrition.
(A10)	21a. clear + and - . No varieg. (V).	FS S	
	20a. Do.	S	M-
	97a. Do.	S	M-
	4a. Do.	S	
	38a. Do.	S	M-
	37a. Do.	S	M-
	113a. Do.	S	M- ✓
	132a. Do.	S	
	80a. Do.	S	
	96a. Do.	S	M-
	133a. Do.	S	
	104a. Do. (1 papilla)	S	THB ₁ - !
	110a. Do.	(B) S	104 Lac - M- }
	106a. Do.	S	
	8a. Do.	S	M-
	75a. Do.	S	M-

Study intensively papillae of (104) (110). Struck - and + to NA slants.

Selective media for fern mutants.

Final Plate out on univalent lactose agar + K_2HPO_4 2g/l +
 lact med -

phosphatidate	1%	+	-
	.1%	-	-
	.05%	-	-
	.01%	+++	+++
	.005%	+++	+++

48 hours.

no differential inhibition!

No Buffer:
 Sod. sulfite 1/2%

Na Benzoate 1%	-	-
.1%	±	±
Na Salicylate 1%	-	-
.1%	±	±

Agar v. soft
 growth only in heavy streak.

Neutral Red. 104%

+++	+++
-----	-----

Background of - changed to yellow.
 Colonies, especially + take up fair amount
 of dye.

Janus Green .04%

++	++
----	----

St. inhibition - cells somewhat reddish
 compared to background. + cells same color as
 background.

Acid Fuchsin.

.4%	+	+
.20%	+++	+++
.1	+++	+++
.05	+++	+++
.02	+++	+++
.01	+++	+++

B = phosphate buffer M/50 7.0

+++	+++
+++	+++
+++	+++
+++	+++
+++	+++
+++	+++
+++	+++

+ colonies generally took up some dye; - did not but decolorized the dye,
 presumably due to alkaline shift.